Novel biomarker panels in diabetic kidney disease

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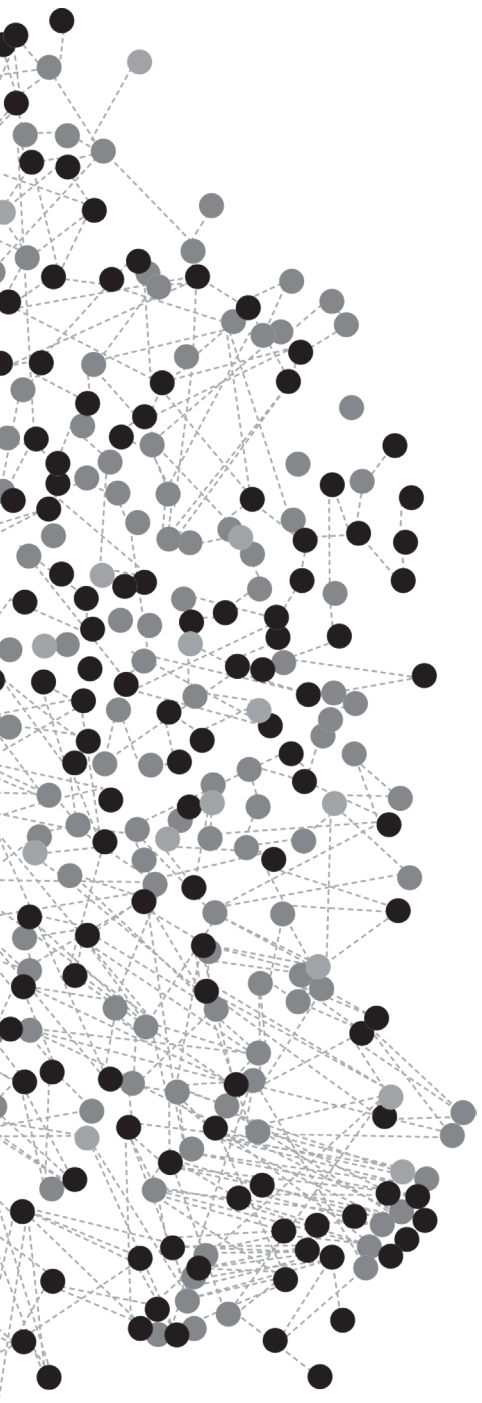

CHAPTER 1

Introduction and aims

Modified from
Prognostic clinical and molecular biomarkers of renal disease in type 2 diabetes
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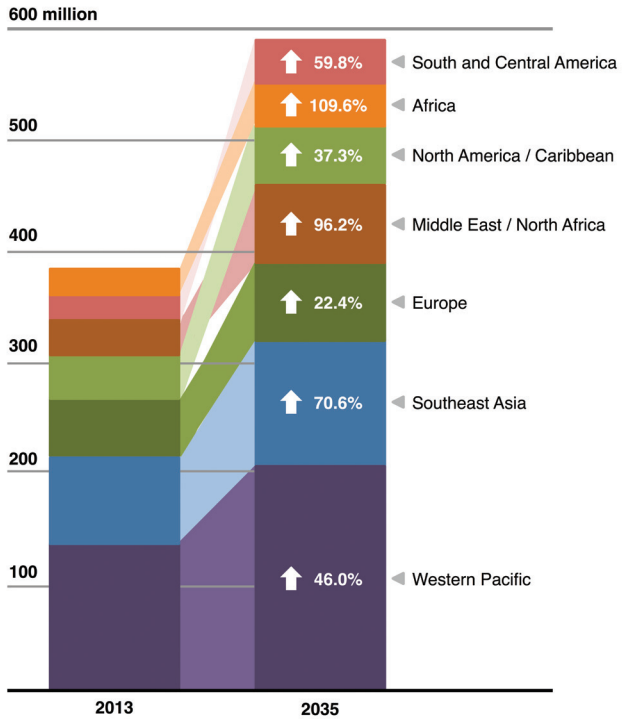
INTRODUCTION

There is an urgency to better identify patients with type 2 diabetes mellitus at early stages of chronic kidney disease (CKD) [1]. Approximately 387 million adults around the world are currently living with diabetes, and due to a relentless increase in the incidence of type 2 diabetes, this estimate is projected to rise to 592 million by 2035 (Figure 1) [2]. Of those patients with type 2 diabetes, 20-40% will ultimately develop diabetic kidney disease (DKD). In addition, type 2 diabetes results in a high cardiovascular morbidity and mortality and a decrease in the patients' health-related quality of life.

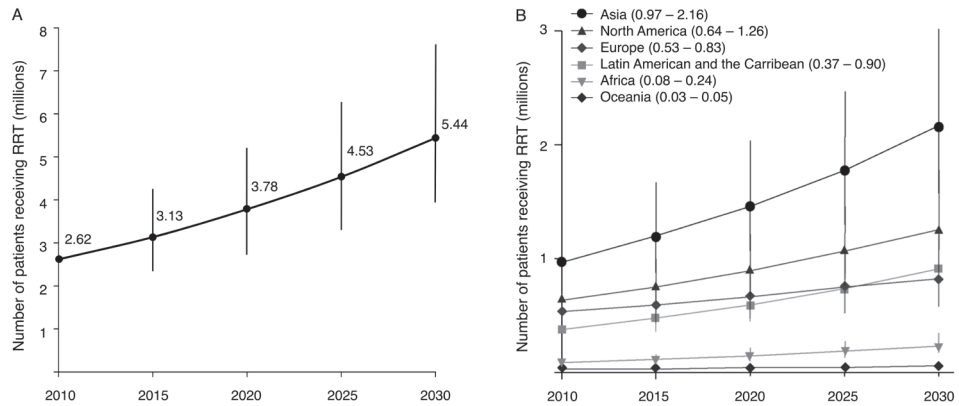
Figure 1. Number of people by region with diabetes in 2013 and projected number of cases in 2035. Adapted from the *IDF Diabetes Atlas* 2013 [2].



DKD, traditionally referred to as diabetic nephropathy, is based in part on the finding of elevated urinary albumin excretion (UAE), progressive decline in glomerular filtration rate (GFR), an increase in systemic blood pressure, and a high risk of kidney failure [3]. DKD is also associated metabolic disturbances. DKD is now the leading cause of end-stage renal disease (ESRD), and accounts for approximately 50% of dialysis and renal transplantation in developed countries [4]. There could be a sharp rise in the prevalence of ESRD over

the next few decades [5], driven by population ageing and the increasing prevalence of diabetes (Figure 2). The costs for renal and cardiovascular related complications are extraordinarily high: costs for renal replacement therapies alone account for 3 to 5% of the total European Union (EU) health care budget and even more in other countries. The United States Renal Data System has reported that for patients aged 65 years and older with both CKD and diabetes, the total Medicare costs have increased more than 11 times in the past decade [6]. Additionally, in a group of patients with type 2 diabetes with early stage CKD in the United States, the 5-year healthcare costs were twice as high among those who progressed to a higher stage of CKD compared to who did not progress, and for patients with stage 3-4 CKD, the costs were more than threefold higher [7]. Thus, there is a strong economic and social imperative to improve the outcomes of type 2 diabetes. Early identification of patients with type 2 diabetes at risk of renal disease can lead to early intervention aimed at reducing the incidence of DKD and ultimately ESRD. There are many stakeholders that can benefit from early identification, number one being the patients themselves, their families, and society.

Figure 2. Estimated number of patients undergoing renal replacement therapy from 2010 to 2030 worldwide (A) and by region (B). 95% CIs shown as error bars. Adapted from Liyanage et al. *Lancet* 2015 [5].



A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [8]. Estimated glomerular filtration rate (eGFR) and detection of albumin in urine (albuminuria) are the classical guideline-endorsed biomarkers for the classification of CKD [9]. These biomarkers are strong predictors of renal disease progression as well as cardiovascular disease and mortality. Reduction in eGFR and detection of microalbuminuria are considered the first clinical signs of renal disease. Reduced eGFR is the consequence of compromised kidney function and substantial loss

and destruction of the glomeruli, and the presence of microalbuminuria already indicates a permeable glomerular basement membrane. Both point to possibly irreversible damage to the kidney. However, renal damage at early disease stages rarely shows clinical characteristics. Therefore, on the everyday clinical level, early stage diagnosis and tailored treatment of DKD are still inadequate. In order to improve patient outcomes and reduce associated health-care costs, timely detection and prevention of progression of renal disease are needed.

Novel biomarker panels can improve identification of renal disease at its early stages. The search for novel biomarker panels to improve the early identification of patients at high-risk for renal disease has been the priority of many researchers for many years. Novel biomarker panels can also have different roles for diagnosis, prognosis, and monitoring by improving risk stratification, help increase our understanding of renal disease pathophysiology, or provide insight into novel therapeutic targets.

Novel biomarker panels as predictors of renal disease

The past decade has produced a large number of papers published on novel biomarkers for renal disease. Many single proteins have been proposed as biomarkers of renal disease in type 2 diabetes and are measured by immunological assays [10-15]. Typically, these biomarkers capture one specific mechanism of disease such as inflammation, fibrosis, or tubular damage. These studies highlight the relevance of single disease mechanisms and provide important insight into the disease etiology. However, type 2 diabetes is a heterogeneous disease involving multiple pathophysiological mechanisms [16]. In theory, the measurement of several biomarkers simultaneously (a multi-marker approach) should improve risk stratification of patients at high risk for adverse events since it is unlikely that a single biomarker may possess useful diagnostic and prognostic power to fully capture the risk of renal disease in type 2 diabetes. Single biomarkers constantly face problems with individual, biological, and analytical variability.

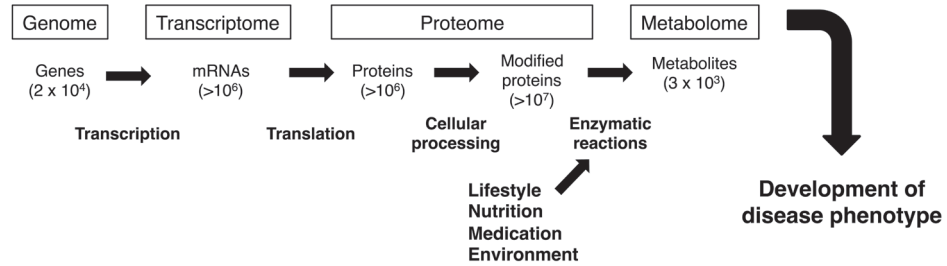
To date, no one, single protein biomarker has been shown to significantly outperform albuminuria or eGFR as predictors of disease progression in longitudinal interventional studies. Alternatively, a panel of clearly defined biomarkers may provide a more robust and reproducible tool as a panel may tolerate changes in single biomarkers without jeopardizing their diagnostic precision and may offer a more realistic picture of disease and its underlying mechanisms. Multiple biomarker approaches are becoming more and more common in literature, though still not as prominent as single biomarker studies. There are however, few prospective studies of multiple biomarkers specifying type 2 diabetes as the cause of renal disease. Some studies consider many biomarkers, but test each biomarker one by one, instead of a combined biomarker panel approach [10,11,15,17]. There are only a few studies in literature that focus on biomarker panels where two or more novel biomarkers are tested in combination to predict renal disease progression [18-21]. Measuring multiple biomarkers at once is becoming more and more

realistic for clinical practice as advancing laboratory techniques with multiplex assays or mass-spectrometry technologies allow the simultaneous measurement of large number of biomarkers with minimal sample volume.

Multiple biomarker panels – Omics platforms

The measurement of multiple biological molecules has advanced significantly over the past years with the introduction of high-throughput omics screening platforms. An omics-based test is defined as an assay composed of, or derived from, multiple molecular measurements and interpreted by a fully specified computational model to produce a clinically meaningful result. Such assays can measure a full spectrum of peptides or metabolites in a short amount of time [22]. The measurement of peptides and metabolites, known as proteomics and metabolomics, have emerged as strong tools in biomarker discovery [22,23].

Figure 3. The conceptual relationship of the genome, transcriptome, proteome, and metabolome.



Adapted from Gerszten & Wang *Nature* 2008 [23].

Proteomics permit the rapid assessment of components of the proteome, which is the complete inventory of proteins (or peptides) present within a biological sample. Biological samples, such as urine, plasma, or serum, can be systematically analyzed with the goal of identifying, quantifying and discerning the function of all observable proteins [24]. In particular, urinary proteomics has gained much attention as a tool for the identification of diagnostic and prognostic biomarkers of renal diseases [25], and may represent an important step forward in the non-invasive diagnosis of renal diseases. Blood-derived proteomics studies are not as common as urine proteomics, a few reasons being that there is large heterogeneity and spread in abundance of proteins in blood and high exposure to proteolytic activity [26], which complicates the analysis of the blood proteome. Metabolomics, i.e. the measurement of low-weight intermediate metabolites (<500Da) and end-products of cellular functions in biological fluids has emerged as another potential

tool to discover novel biomarkers for renal disease. The metabolome can be viewed as the down-stream integration of biological information of the genome, transcriptome, proteome, and overall enzymatic reactions of an individual [23], and therefore enables the detection of short and long-term physiological or pathological changes occurring in chronic diseases. Omics-based approaches hold promise for new diagnostic tests, better understanding of pathogenesis, and evolution of a disease.

Novel biomarker panels for predicting response to therapy

Despite guideline recommended therapy for reduction of hypertension and albuminuria, not all patients with diabetes respond well to first line therapy intervening in the renin-angiotensin-aldosterone system (RAAS) [27]. Furthermore, there is large intra- and inter-individual variability in response to RAAS inhibiting therapy [28]. Many patients still have significant residual proteinuria [29]. In addition, a proportion of patients experience off-target effects [30-32], which may contribute to progressive renal function loss. The reasons behind these individual differences in response to therapy are unknown, and may be related to differences in systemic vs. renal tissue-specific renin-angiotensin system activity [33], dietary sodium consumption [34], or difference in genetic make-up [35, 36], among other factors. One strategy to improve the current state-of-the-art treatment is to tailor drug therapy by using a complementary approach to attribute drug response variability to individual variability in underlying molecular mechanisms involved in the progression of disease. On one hand, the interplay of different processes such as inflammation, fibrosis, angiogenesis, or oxidative stress, appears to drive disease progression, but the individual contribution of each process varies. On the other hand, drugs address specific targets and thereby interfere in certain disease associated processes. At this level novel biomarker panels may help gain insight into which specific pathophysiological processes are involved in an individual followed by a rational assessment whether a specific drug's mode of action indeed targets the relevant process. In this context, novel biomarker panels can be used to identify a group of patients more likely to beneficially respond to therapy. This may reduce this inter-individual variation in response to medication. However, studies evaluating whether novel biomarker panels can be used as predictors of response to therapy have only been marginally explored.

Novel biomarker panels for monitoring drug effect

A third option for using novel biomarker panels are to use changes in biomarkers to monitor the effect of therapy. This is important because it allows one to make a better estimate of the drug effect after the individual is exposed to the drug for a short period of time. In addition, results of such studies may also provide insight into the mechanisms through which drugs exert renoprotective effects and yield novel biomarkers to monitor response to therapy in patients with type 2 diabetes and DKD. Studies evaluating whether changes in novel biomarker panels can be used as predictors of renal disease are limited in existing literature.

AIMS OF THIS THESIS

This thesis examines several different approaches of utilizing novel biomarker panels in diabetic kidney disease that can be used to predict disease progression, predict response to therapy, or monitor effects of therapeutic intervention.

Part 1. Novel biomarker panels for predicting disease progression

Part 1 begins by investigating the predictive ability of novel biomarker panels for the progression of renal disease in patients with type 2 diabetes. **Chapter 2** evaluates the ability of a panel of novel, assay-based biomarkers representing different disease pathways to improve prediction of renal function decline in type 2 diabetes, and to assess their combined predictive performance of accelerated renal function decline. In **Chapter 3**, proteomic analysis is used to identify plasma peptides associated with transitioning in stage of albuminuria in hypertension or type 2 diabetes, and examines whether two classifiers, one for hypertension and another for type 2 diabetes, are able to predict the transition of stage of albuminuria. In **Chapter 4**, metabolomics is performed to investigate the predictive ability of urine and plasma metabolites for the progression of renal dysfunction in patients with type 2 diabetes, and tests whether the metabolites are specific to type 2 diabetes by assessing the metabolites in individuals with hypertension without type 2 diabetes.

Part 2. Novel biomarker panels for predicting response to therapy and monitoring drug effect

Part 2 examines novel biomarker panels for predicting response to therapy in diabetes mellitus and monitoring the effect of therapeutic intervention. **Chapter 5** first discovers and then validates a serum metabolite classifier that predicts response in albuminuria to angiotensin receptor blocker (ARB) therapy in patients with diabetes mellitus. Chapter 5 further integrates the identified metabolites in a molecular process model capturing disease pathophysiology at the interface of drug mechanism of action to decipher the underlying molecular processes driving albuminuria response to ARB. **Chapter 6** assesses the correlation between a previously discovered metabolomics signature of diabetic kidney disease and eGFR in patients with type 2 diabetes and nephropathy, and evaluates the effect of atrasentan on these urinary metabolites.

This thesis ends by discussing future perspectives for using novel biomarker panels to improve on the status quo of choosing drugs for treatment of DKD in patients with type 2 diabetes and as a strategy to guide personalized medicine.

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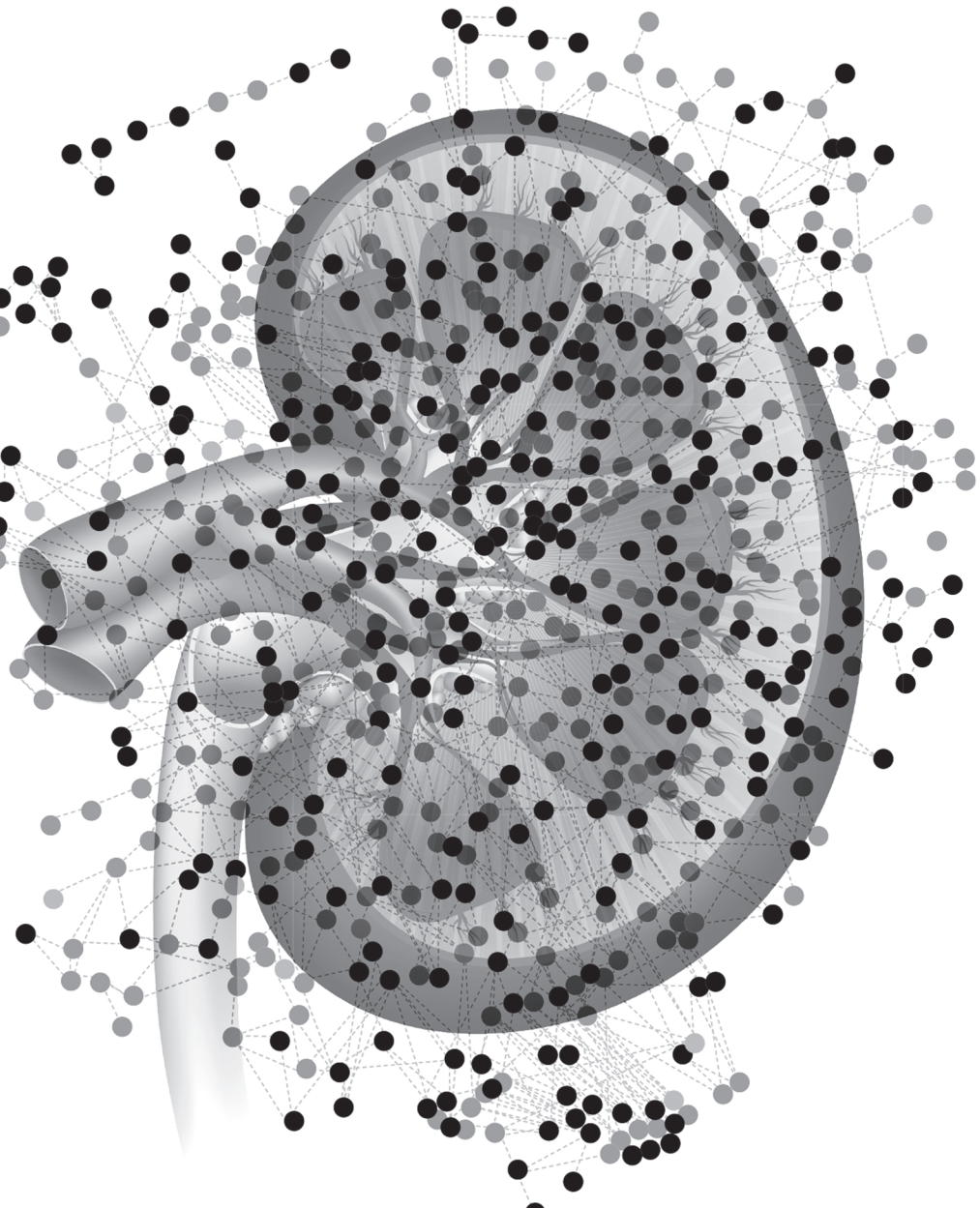

PART 1

Novel biomarker panels for predicting disease progression